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EDITORIAL

SALMONELLOSIS AND MILK HYGIENE

A LITTLE over 20 years ago the preface to a report on the study of 100 outbreaks of food poisoning stated that: "Progress on that side of the inquiry in which it was desired to deal effectively with the paths of infection through domestic and agricultural animals has halted in spite of much effort, for want of better facilities in this country for systematic studies of comparative pathology, but it is hoped that in the early future the work can be extended successfully in this direction."

It was clear at the time this was written that, although the work on Salmonella bacteria was making progress, especially as regards their behaviour in the laboratory, there was a serious lack of information regarding their natural habitat and reservoirs.

Knowledge on these points has been greatly extended and made more readily available during the 20 years that has intervened. Recently the domestic fowl has been shown to harbour and suffer from infection with many more types of Salmonella than was realised at one time. One of the clearest types of host specifically in this group of bacteria is shown to be that of Salmonella dublin for cattle.

However, workers were of the opinion that its incidence as a cause of disease in cattle was relatively low in these islands and considered the problem to be one of public health rather than one of agricultural economics. It is now realised that this conception is far from true, and the work of Field reported in this journal shows how widespread the infection is in certain parts of Wales.

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Although the incidence of clinical cases and of carrier animals is high, no outbreak of food poisoning traceable to cattle was reported in Wales during the period under survey.

In spite of the absence of official records of cases of food poisoning which could be attributed to the infection occurring in West Wales during the period under Field's review, one cannot accept such negative evidence as indicating that certain alimentary and other disturbances in humans which occur from time to time in either local or widespread epidemics have not in some cases been due to the presence of S. dublin in food or milk.

It is, therefore, of importance to realise the truth of Field's statement that "The hygiene of milk production during the summer months is the main factor governing the number of outbreaks of milk-borne food poisoning."

GENERAL ARTICLES

A SURVEY OF BOVINE SALMONELLOSIS IN MID AND WEST WALES

By H. I. FIELD

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Five hundred and forty blood samples from adult female bovine animals, received in the laboratory for examination for agglutinins to *Br. abortus*, have at the same time been tested for agglutinins to *S. dublin*. In addition, 80 samples from cattle entering an abattoir for slaughter have been submitted to test. The examination of these sera did not constitute as critical a test of the titres of normal animals as did the examination of the herd, for no history was available of either individual animals or the herds of which they were members. It is therefore possible that at least some of these animals may have been exposed to previous infection with *S. dublin*. Of the 620 samples, 606 (97.6 per cent.) had no flagellar agglutinins or agglutinated *S. dublin* antigen at a titre of 1/80 or less. Twelve samples had titres of 1/160 and two of 1/320. Somatic agglutinins to a titre of 1/40 or less were shown by 591 samples (95.3 per cent.). Twenty-three samples had titres of 1/80. The remaining six agglutinated *S. dublin* antigen at a titre of 1/160.

These results are, broadly speaking, in agreement with those obtained in the herd test, over 95 per cent. of the samples being either negative or having flagellar titres less than 1/160 and somatic titres less than 1/80.

By contrast to the test in the herd with no known history of salmonella infection, a table is given (Table 6) showing the result of a test in a herd with a known history of infection in both adult cows and calves. The differences in the agglutinating titres are striking. Of the 23 calves on the affected farm, eight had flagellar titres ranging from 1/80 to 1/1280; two had somatic titres of 1/80, and one a somatic titre of 1/640. In cattle of the age groups 3-5 years, nine animals had flagellar titres in the range of 1/160 to 1/1280, and seven animals somatic titres in the range 1/320 to 1/1280.

The Agglutinating Titres of Clinically Affected Adult Cattle

A total of 26 blood samples taken from adult bovines during a clinical attack of *S. dublin* infection has been submitted to agglutination tests. The results are shown in Table 7. Twenty-one of the 26 animals had agglutinating titres within the range of those found in normal healthy cattle. The sample from one animal with a flagellar titre of 1/320 was taken on the eighth day of illness, by which time, as will be shown later, infection has stimulated the production of antibodies. It is not known at what stage of illness the remaining four samples showing titres higher than normal were taken.

The Rate of Antibody Production during Illness

The course of *S. dublin* infection in adult cattle is so rapid, and the mortality rate so high, that it is extremely difficult to obtain daily samples of serum from

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TABLE V

Agglutination titres of cattle of various age groups to S. dublin

NON-INFECTED HERD

Group	"H" agglutinins										"O" agglutinins									
	Neg.	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	Neg.	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Total
Calves 0-6 m.	18	3	1	—	—	—	—	—	—	—	9	12	1	—	—	—	—	—	—	22
Calves 6-12 m.	4	1	—	—	1	—	—	—	—	—	2	3	—	1	—	—	—	—	—	6
Cattle 1-3 years	9	8	14	6	3	1	—	—	—	—	2	17	20	2	—	—	—	—	—	41
Cattle 3-5 years	3	4	10	5	3	—	—	—	—	—	1	12	9	3	—	—	—	—	—	25
Cattle 5-8½ years	3	1	7	4	2	—	—	—	—	—	1	7	7	2	—	—	—	—	—	17
																		TOTAL ...		111

TABLE VI

Agglutination titres of cattle of various age groups to S. dublin

INFECTED HERD

Group	"H" agglutinins										"O" agglutinins									
	Neg.	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	Neg.	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	Total
Calves 0-6 m.	12	1	2	—	1	1	2	—	4	—	17	2	—	1	2	—	—	1	—	23
Cattle 1-3 years	11	4	3	—	—	—	—	—	—	—	2	6	8	2	—	—	—	—	—	18
Cattle 3-5 years	7	1	3	2	—	2	—	2	5	—	—	3	9	1	2	—	3	3	1	22
Cattle over 5 years	11	2	—	3	1	1	—	—	—	—	—	3	10	4	1	—	—	—	—	18
																		TOTAL ...		81

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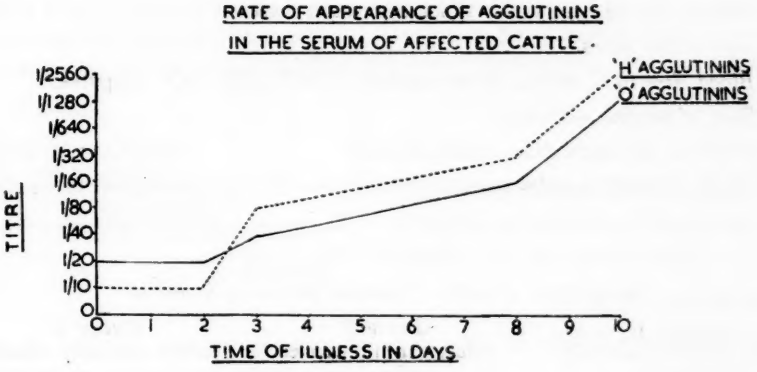
any one animal for a period long enough to form a strictly accurate picture of the rate of development of agglutinins in response to infection. By utilising data derived from a number of affected animals, however, a reasonable indication of

FIGURE 1.



the rate of antibody production can be obtained. Fig. 1 shows graphically the rate of development of antibody, based on information gained from the following cases :—

	"H"	"O"
Animal No. 1		
Blood titre—2nd day of illness	1/10	1/20
Blood titre—8th day of illness	1/320	1/160
Animal No. 2		
Blood titre—3rd day of illness	1/80	1/40
Animal No. 3		
Blood titre—8th day of illness	1/320	1/80
Animal No. 4		
Blood titre—10th day of illness	1/2560	1/1280

Samples have been obtained from two animals on the eighth day of illness and in both cases the flagellar titre was 1/320. The data obtained suggests that a blood sample examined before the fifth day of illness would have no diagnostic value. A blood sample taken on the seventh or eighth day would show a titre higher than those of normal animals. The greatest value of the agglutination test as an aid

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to diagnosis in affected animals would be, however, when used retrospectively, a rise in titre being diagnostic of infection with *S. dublin*.

The Rate of Antibody Production following Vaccination

It is interesting to compare with these results the reaction of an adult cow following vaccination with a heat-killed *S. dublin* vaccine. The details of the experiment are as follows:—

Subject.—Blue roan Welsh Black-Shorthorn heifer.

Age.—Two years.

Blood titre to S. dublin at vaccination.—"H" 1/20; "O" negative.

Dose of vaccine.—5 c.c.

Route of administration.—Subcutaneous.

Result.—Blood samples were taken daily for 21 days subsequent to vaccina-

TABLE VII

Blood titres of cattle of various groups to S. dublin

Group 1 Adult animals clinically affected			Group 2 Adult chronic carrier animals				Group 3 Calves clinically affected or exposed to infection		
No.	"H"	"O"	No.	"H"	"O"	Time since affected	No.	"H"	"O"
1	1/40	1/20	1	1/640	1/320	3 m.	1	1/160	Neg.
2	1/80	1/40	2	1/1280	1/320	3 m.	2	1/320	Neg.
3	1/10	1/10	3	1/1280	1/160	3 m.	3	1/320	Neg.
4	1/160	1/10	4	1/640	1/320	21 m.	4	1/320	Neg.
5	1/160	1/10	5	1/1280	1/320	15 m.	5	1/1280	Neg.
6	1/160	1/80	6	1/1280	1/640	15 m.	6	1/1280	1/10
7	1/10	1/20	7	1/1280	1/320	15 m.	7	1/640	1/20
8	1/40	1/40	8	1/1280	1/80	25 m.	8	1/1280	1/20
9	1/320	1/80	9	1/640	1/640	15 m.	9	1/320	1/40
10	1/40	1/160	10	1/640	1/320	15 m.	10	1/1280	1/40
11	Neg.	Neg.	11	1/1280	1/640	15 m.	11	1/1280	1/80
12	Neg.	Neg.	12	1/2560	1/160	15 m.	12	1/1280	1/80
13	1/40	1/80	13	1/640	1/160	24 m.	13	1/1280	1/160
14	1/10	1/20	14	1/1280	1/320	24 m.	14	1/1280	1/640
15	1/160	1/40	15	1/2560	1/640	1 m.			
16	1/20	1/10	16	1/81960	1/640	1 m.			
17	1/10	1/80	17	1/163920	1/1280	1 m.			
18	1/10	Neg.	18	1/1280	1/1280	15 m.			
19	Neg.	1/10							
20	1/10	Neg.							
21	1/20	1/20							
22	1/20	1/20							
23	1/40	1/20							
24	1/80	1/80							
25	1/40	1/40							
26	1/20	1/40							

tion and thereafter at intervals. The blood titre commenced to rise on the fourth day following vaccination and remained level after the sixth day. On the eighth day a second dose of 5 c.c. of vaccine was given subcutaneously. A second rise in the blood titre commenced on the ninth day and reached its peak on the 13th day after the commencement of the experiment when the "H" (flagellar) titre was 1/1280 and the "O" (somatic) titre 1/160. The response of this animal to vaccination is shown in Fig. 2.

The Agglutinating Titres of Adult Carrier Animals

Table 7 shows the agglutinating titres of 18 adult animals at periods varying from 1-25 months after clinical recovery. The lowest flagellar titre in the serum of any animal in this group is 1/640 and the lowest somatic titre 1/80. A point

FIGURE 2.

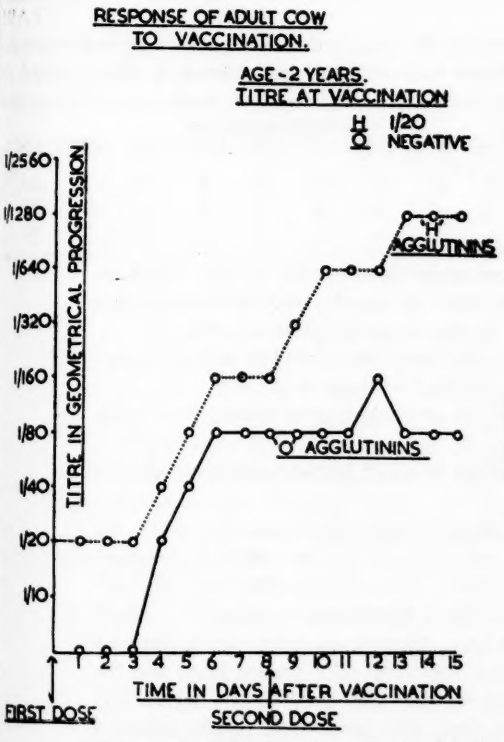
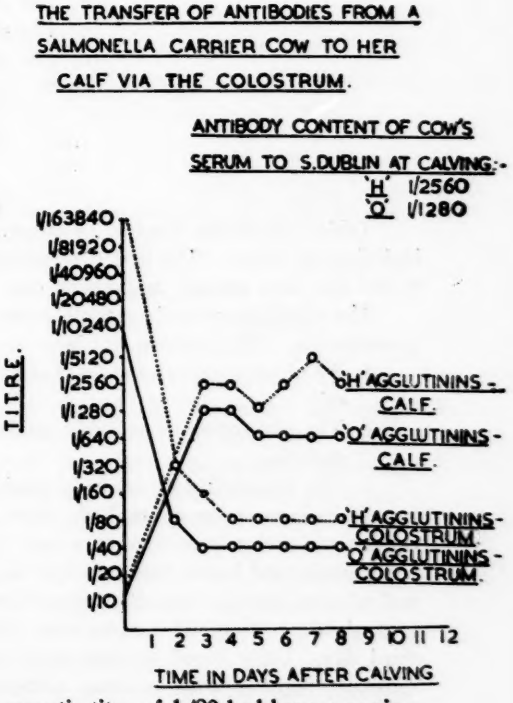


FIGURE 3.



of importance is that the animal with the somatic titre of 1/80 had been a carrier for 25 months.

A composite table has been prepared, Table 8, in which the titres of a normal herd of cattle, of affected animals and of carrier animals are available for comparison. In comparing the titres of normal with carrier animals, it will be seen that there is a larger gap between the flagellar titres of normal animals than there is between the respective somatic titres. The highest flagellar titre in the normal

cattle is 1/160 and the lowest titre in carrier animals 1/640. The highest somatic titre in normal cattle is 1/40 and the lowest in carrier animals 1/80. The difference in the flagellar titres allows of a clear line of demarcation at 1/320, animals with titres below this being considered as negative while animals with titres above this should be regarded as positive for the carrier state.

The Agglutinating Titres of Infected Calves

A study of the blood titres of infected calves shows that the following variations in agglutinating titres may be encountered :—

1. High "H" and "O" agglutinins.
2. High "H" and low "O" agglutinins.
3. High "H" and no "O" agglutinins.

TABLE III

(a) *The serum titres to S. dublin of a herd of cattle not known to be exposed to infection*
(b) *The serum titres of adult cattle during a clinical attack of S. dublin infection*
(c) *The serum titres of adult cattle recovered from a clinical attack of S. dublin*

Group	"H" agglutinins										"O" agglutinins
	Neg.	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	Neg.
(a)	37	17	32	15	9	1	—	—	—	—	15
(b)	3	6	4	6	2	4	1	—	—	—	4
(c)	—	—	—	—	—	—	—	5	9	1	—

Table 7 shows the titres of 14 calves, all under four months of age. All have high flagellar titres. The first five animals have no somatic agglutinins while the second five have somatic agglutinins only in the range of 1/10 to 1/40.

The significance of the variations in titre found in calves demanded further investigation. The problem has been investigated in three ways :—

1. By observations on the transmission of antibody from a carrier cow to her calf.
2. The stimulation of antibody production in a calf by the feeding of infected material.
3. The stimulation of antibody production in calves by vaccination.

The transmission of antibody from a carrier cow to her calf.—A pregnant carrier cow with a high blood titre was selected. Immediately after birth the calf was muzzled and before being allowed to suckle a blood sample was taken. Blood and colostral samples were taken from the dam. All were examined for agglutinins to *S. dublin*. No blood samples were taken from the calf between birth and the third day. Daily blood samples were taken from the third to the eighth day inclusive, together with colostral samples from the dam. The results obtained are shown graphically in Fig. 3. At birth the serum of the calf showed incomplete agglutination at a dilution of 1/10. By the third day of life, the serum of the calf contained both "H" and "O" agglutinins to a level equal to those in the serum of the dam.

The stimulation of antibody production by the feeding of infected material.—An experiment was designed in an attempt to reproduce clinical symptoms of salmonellosis in calves by the daily administration of broth culture of *S. dublin*

in the milk. One of the calves did not show any sign of illness although culture was added to the milk supply from the fifth to the 32nd day of life. The blood titre remained consistently negative. On the 33rd day, 25 c.c. of a mixture of bile and intestinal contents obtained at *post-mortem* of an affected adult animal was given *per os*. The calf remained fit and well. Two days subsequent to the administration of the infected material the serum of the calf showed a flagellar titre of 1/40 and incomplete somatic agglutination at 1/40. By the fifth day the flagellar titre had risen to 1/1280 while somatic agglutination was incomplete at 1/80 (Fig. 4).

The stimulation of antibody production by vaccination.—Two calves have been used for vaccination experiments, one aged eight days at vaccination and the second 35 days. The vaccine used was the same as in the adult cow.

TABLE III

be exposed to infection with S. dublin.
infection.
ck of S. dublin infection.

	"O" agglutinins								
Neg.	1/10	1/20	1/40	1/80	1/160	1/320	1/640	1/1280 & over
15	51	37	8	—	—	—	—	—
4	5	6	5	5	1	—	—	—
—	—	—	—	1	3	7	5	2

Details of the two experiments follow:—

Calf No. 1

Age at vaccination—8 days.
Blood titre at vaccination—" H " negative; " O " negative.
Dose of vaccine—5 c.c.
Route of administration—Subcutaneous.

Agglutinins to *S. dublin* were demonstrable on the fourth day following vaccination and the peak titre of 1/2560 was reached on the sixth day. These agglutinins were flagellar only, no somatic agglutinins appearing following vaccination. On the eighth day a second dose of 5 c.c. of vaccine was given subcutaneously but the calf did not respond by the production of further agglutinins.

Calf No. 2

Age at vaccination—35 days.
Blood titre at vaccination—" H " negative; " O " negative.
Dose of vaccine—5 c.c.
Route of administration—Subcutaneous.

Flagellar agglutinins began to appear in the serum on the third day following vaccination and reached a peak titre of 1/2560 on the seventh day. A second dose of vaccine on the tenth day following the primary dose failed to cause a rise in the level of flagellar agglutinins. No production of somatic agglutinins followed the primary dose of vaccine but on the fourth day following the second dose incomplete agglutination occurred at dilutions of from 1/10 to 1/40. These agglutinins disappeared rapidly and were not demonstrable five days later. The results obtained in the vaccination of the two calves are shown in Fig. 5. The

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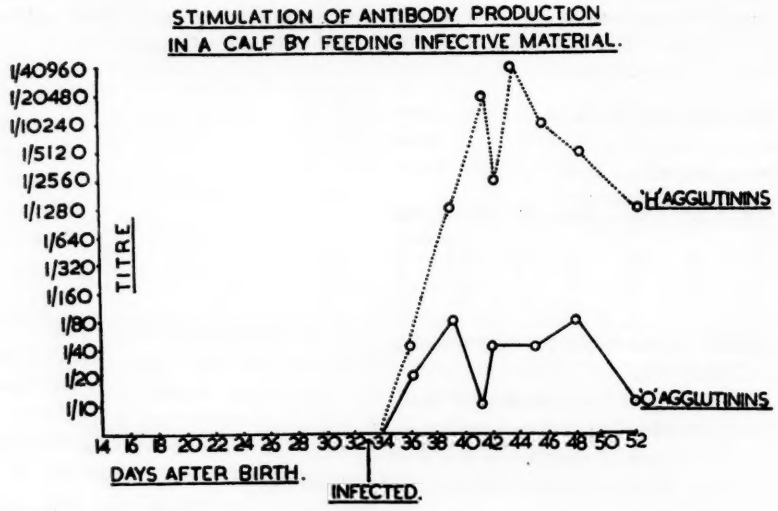
experiments indicate that a maximum response to the flagellar antigens of *S. dublin* occurs in calves following a primary stimulus, whereas in an adult cow a secondary stimulus evoked a secondary response. Together with the observations made on calves exposed to natural infection, the experiments show that young calves, while producing flagellar agglutinins, do not produce somatic agglutinins. Where both flagellar and somatic agglutinins are present in the serum of a young calf, such agglutinins are passively acquired from the dam via the colostrum. Calves of 5-6 weeks respond feebly to the stimulus of the somatic antigens of

FIGURE 4.



S. dublin. The ability to produce somatic agglutinins appears to be fully developed at 3-4 months. Calf No. 14, Table 7, Group 3, was 3½ months old when exposed to infection.

Epidemiology

The source of infection to cattle has not been satisfactorily determined. Some evidence has, however, been obtained offering a contribution to the problem and indicating lines of enquiry to be followed in future work. The facts presented are based on personal observation supported where desirable by observations made by other workers.

Several points of importance have been recorded fully in previous sections. Only a brief reference will be made to these in the present section.

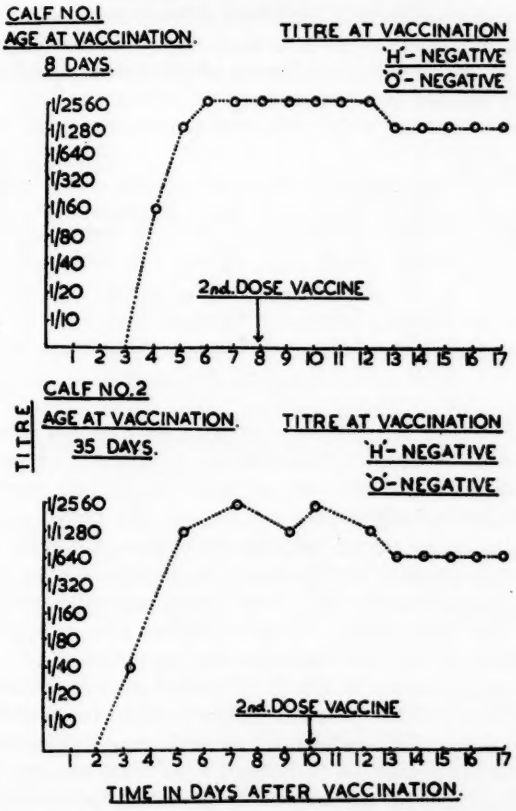
1. *The disease in calves.*—Two outbreaks of disease due to *S. dublin* infection have been investigated in calves. An examination of one herd showed the presence of adult carrier animals with a history of previous illness. The term adult carrier animal refers specifically to animals with high blood titres and shown to be excreting the organism in the fæces.

The transmission of infection from such carrier animals to calves has been shown conclusively to occur (see case No. 1: Protocols of representative outbreaks). Standfuss *et al.* (1933) also noted that calves allowed to suckle cows excreting the organisms in the fæces became ill.

Apart from this specific outbreak, evidence has been obtained from a number of other farms that where carrier animals are present, calves born on such farms are exposed to infection, and further, that such calves do not necessarily have to

FIGURE 5.

RESPONSE OF CALVES TO VACCINATION.



be born from carrier cows. Not all calves exposed to infection develop clinical symptoms of disease. This has been confirmed by the feeding of infective material to an experimental calf. The only reaction of this animal was the production of agglutinins.

On the other hand, an examination of the second herd in which the disease was present among calves failed to reveal any adult carrier animal. Similar findings have been reported by other workers. Miessner and Koebe (1931) when

investigating outbreaks in calves, found adult carrier animals in some herds, but failed to do so in all cases. Craig *et al.* (1941) also failed to find adult carrier animals when investigating an outbreak in calves. These observations indicate that outbreaks of salmonellosis may occur without the presence of adult carrier animals in the herd.

2. *The Persistence of Infection in Calves.*—A number of calves have been periodically tested subsequent to recovery from a clinical attack of *S. dublin* infection. The blood serum has been subjected to an agglutination test and the fæces cultured for the specific organism. Of seven calves examined in this way, not one has continued to excrete the organism in the fæces. Bosworth and Lovell (1931) reported similar findings. A significant difference has, however, been found in the blood titres of individual calves at these tests. To illustrate this point, the results are given of the periodic examination of two calves, members of the same herd and clinically affected at the same time.

Calf No. 1				*Calf No. 2*			
		Blood titre				Blood titre	
Age	Fæces	"H"	"O"	Age	Fæces	"H"	"O"
3½ m.	*S. dublin*	1/1280	1/640	1 m.	*S. dublin*	1/1280	Neg.
5½ m.	Neg.	1/320	1/80	2½ m.	*S. dublin*	1/1280	Neg.
7½ m.	Neg.	1/160	1/20	5½ m.	Neg.	1/640	Neg.
8½ m.	Neg.	1/160	1/10	6½ m.	Neg.	1/1280	Neg.
9½ m.	Neg.	1/160	1/10	7 m.	Neg.	1/1280	1/10
12½ m.	Neg.	1/40	1/10	10 m.	Neg.	1/640	1/20

The reaction of Calf No. 1 is in accordance with normal expectations in an animal making a clinical recovery from disease. Calf No. 2 was severely affected clinically, showing symptoms not only of dysentery but of involvement of the carpal and tarsal joints. Although the fæces of this animal were consistently negative subsequent to the second test, the persistence of flagellar agglutinins to a high titre suggested a possible localisation of the bacteria in some organ or tissues. When the animal was 16 months old, blood, fæces and urine samples were taken and examined. The blood serum showed a flagellar titre to *S. dublin* of 1/160 and a somatic titre of 1/10. The organism was not recovered from the fæces but was recovered from the sample of urine. It seemed possible, therefore, that some calves recovered from calfhood infection might continue to carry the infection as adults. In order to test this possibility, a survey is now in progress to determine whether salmonella organisms can be isolated from cattle entering an abattoir for slaughter. It is sufficient for the purpose of this article to say that *S. dublin* and *S. typhi-murium* have been isolated from such animals. The finding that not one of the animals yielding a positive culture had a significant blood titre to the isolated organism suggests that such animals had not suffered from a clinical attack of salmonellosis as adult animals.

3. *The disease in adult cattle.*—The great majority of clinical cases in adult cattle occur during the warmer months of the year. In most herds only one animal is clinically affected, although other animals may be suffering from transient

infection. In other herds several animals may be clinically affected either concurrently or at intervals, while occasionally atypical cases occur showing no clinical symptoms but giving rise to the carrier state. Animals recovered from clinical infection remain carriers and continue to excrete the organism in the fæces. A *post-mortem* examination of such a carrier animal showed that the residual foci of infection included the gall bladder, spleen, and all the lymph glands draining the liver and gastro-intestinal tract.

Four herds containing such carrier animals have been kept under observation. In all of them the carrier animals mixed and grazed freely with the other cattle. It is interesting, therefore, to consider briefly the history of these herds during the period of observation.

Herd No. 1.—One adult carrier animal present in the herd from June, 1946, to September, 1947. Periodic fæces examinations were always positive for *S. dublin.* No illness occurred among adult cattle in the herd during the period of observation.

Herd No. 2.—Seven adult carrier animals present in herd from October, 1945, to March, 1946. Three animals were then disposed of but four remained in the herd until October, 1947. The fæces of carrier animals were always positive at periodic tests. Infection occurred in calves during the winters of 1945-46 and 1946-47. No clinical cases appeared in adult cattle.

Herd No. 3.—During a period of 21 months a carrier animal was present in the herd but no clinical case occurred in adult cattle. Periodic samples of fæces from the carrier cow were invariably positive.

Herd No. 4.—Two adult carriers were in the herd for 24 months. No clinical case appeared amongst the remaining adult cows for 22 months. A fatal case then occurred in a newly-introduced cow. The fæces of the two carrier cows yielded *S. dublin* at all tests.

It is apparent from a consideration of the history of these four herds that, while acting as a source of infection to calves, the presence of carrier animals did not result in further clinical cases in adult animals, with the exception of the case in Herd No. 4, despite the fact that the carriers were consistently excreting the organisms in fæces.

The Survival of S. dublin in Fæces and Water

A knowledge of the period of survival of salmonella organisms in fæces is of fundamental importance to a study of the epidemiology of the disease. Henning (1939) showed that fæces containing *S. dublin* dried in the incubator for 48 hours and kept in the laboratory yielded *S. dublin* for 1,069 days. At this stage attempts at recovery were discontinued.

During the course of the present work, fæces collected from carrier animals and stored in tins have been kept in the laboratory and cultured periodically. *S. dublin* was recovered from one sample after 159 days.

Similar samples have been deposited on pasture. One sample, deposited in January, 1947, yielded *S. dublin* 73 days later, when observations were discontinued. Another, deposited during the summer months, was positive 119 days later.

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Observations have also been made on the survival of *S. dublin* in water. In three out of four tubes each containing 20 ml. of tap water and inoculated with a loopful of fæces from a carrier animal, viable organisms were still present after 87 days.

Many of these findings are difficult to reconcile. It is clear that carrier animals retained in herds act as reservoirs of infection for calves while adult cattle when exposed to constant infection may remain clinically normal. Nevertheless, they do not develop an immunity as judged by agglutination titres. Against this is the established fact that clinical cases in adult cattle and calves occur in herds in which no carrier animals can be demonstrated and that over 70 per cent. of such cases in adult cows are acute and rapidly fatal.

The significance of the latent carrier remains to be determined. It may well prove that such animals are an essential link in the epidemiology of bovine salmonellosis.

This does not mean that there are no other possible sources of infection. Previous workers have reported the isolation of salmonella organisms from rats. Thus Savage and White (1923) examined 96 rats from two slaughterhouses and isolated *S. enteriditis* from six, while Khalil (1938) isolated organisms of the salmonella group from 7.3 per cent. of 750 wild rats trapped in Liverpool. Of the 89 strains isolated, 45 belonged to the *S. enteriditis* and 40 to the *S. typhi-murium* types.

During the present observations, only 30 rats have been examined. The animals were caught in traps on two farms, one in Cardiganshire and the other in Carmarthenshire. On the first farm clinical cases of salmonellosis had occurred in both adult cattle and in calves. Cases had also occurred on a farm adjoining the second farm. *S. dublin* was isolated from the liver and/or intestinal contents of six of the 30 rats examined. Positive cultures were obtained from each batch of rats, i.e., from the Cardiganshire and Carmarthenshire specimens. This indicates one alternative possible source of infection. There may well be others. Further work is obviously necessary on this aspect of the problem.

The Relationship between Salmonellosis of Cattle and Outbreaks of Food Poisoning among the Human Population

In a consideration of a bovine disease of this nature, due to organisms which may be associated with disease in man, it is important that attention should be directed to those aspects of the disease having a bearing on the transmission of infection to the human population.

The course of the clinical disease in affected cattle is usually so short that it must be a rarity for an actively infected animal to be sent to an abattoir for emergency slaughter. Further, it seems improbable that such an animal would be passed for human consumption at subsequent meat inspection. That exceptions do occur is shown in the report by Scott (1936) of a food poisoning outbreak traced to the carcass of an animal from all parts of which *S. typhi-murium* was isolated. Observations made by the writer on cattle entering an abattoir for

slaughter show that the methods of meat inspection as practised in this country will *not* detect carrier animals. It is probable, however, that such animals do not represent an important source of infection to the human population, for it is clear from a study of public health reports in this country that in the majority of outbreaks of food poisoning traceable to cattle, the source of the infection has been contaminated milk. In some cases the milk has come from herds containing clinically affected animals, in others from herds containing healthy carrier animals. From the description of the clinical symptoms in salmonella infection, it is clear that the first recognisable symptoms of illness are an elevated temperature, depression and fall in milk yield. Enteric symptoms do not develop until later. During this early stage the organism is present in the blood stream, and *ipso facto*, is present in the milk. The observant stockman realises that the animal is not normal but has not the knowledge to appreciate the significance of these early symptoms. He notes the depressed milk yield but without doubt in this early stage the milk is added to that of the remainder of the herd. This would appear to be the stage when transmission of infection to human beings is most likely to occur. By the time enteric symptoms have appeared, the milk yield is usually negligible, and is either not drawn or the small quantity obtained is discarded. This stage, nevertheless, is not without its danger, for the fæces of the animal now contain the organisms and contamination of milk by fæces may act as an equally potent source of infection.

The source of infection of milk from the healthy carrier animal is not so clearly defined. Observations on carrier animals made during this survey have been confined to carriers of *S. dublin* but there is every reason to believe that animals recovered from *S. typhi-murium* infection also remain carriers. Rasch and Lorenzen (1935) reported a carrier animal which excreted *S. typhi-murium* in fæces for more than five months.

The evidence pointing to the excretion of organisms in milk by carriers is conflicting. While most investigations of milk-borne epidemics have resulted in the fæces of the carrier animal being incriminated as the source of the infection, Standfuss *et al.* (1932), who made a series of observations on six excretors, detected the organisms in milk and urine on a number of occasions. Clarenburg (1931), while agreeing that milk might become infected from fæces, was also of the opinion that there might be an actual infection of the udder.

Observations made on the carrier animal and recorded in Case No. 4 of the protocols show that in this animal, using precautions to ensure that the samples were not contaminated from extraneous sources, salmonella in small numbers were isolated from milk drawn from the udder. One has, however, to differentiate between organisms excreted in the milk and organisms found in milk drawn from the udder, for clearly, the organisms may have gained access to the teat canal from the outside. Whatever the explanation may be, it is clear that the technique adopted by a milker could do nothing to prevent organisms gaining access to the milk, if these were already present in the udder. The *post-mortem* findings that the spleen is a residual focus of infection in the carrier animal suggests that organisms may from time to time pass into the blood stream and so into the milk.

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The death *in utero* of the fœtus in the carrier animal in Case No. 1 of the protocols must either have been due to a hæmatogenous infection or a localisation of infection in the uterus. If the former source is correct, then again it is apparent that the organism could be excreted in milk.

While none of these findings are conclusive, the evidence favours a view that the milk of carrier animals may from time to time contain salmonella organisms. The evidence is conclusive, in so far as carriers of *S. dublin* are concerned, that these animals constantly excrete the organism in the fæces. Unless careful hygienic measures are enforced at milking, organisms from this source may readily act as a source of contamination.

Despite the high incidence of clinical cases and of carrier cows, no outbreak of food poisoning traceable to cattle has been reported in West Wales during the period covered by this survey. It is also of interest to note that only 11 cases, or outbreaks, of food poisoning due to *S. dublin* were recorded in the whole of England and Wales during the five years, 1940-44 inclusive (Wilson and Miles, 1946).

It must be borne in mind that other salmonella organisms are not pathogenic for human beings in the sense that *S. typhi* is, for whereas minute amounts of infective material containing this organism may cause typhoid fever, experiments on volunteers have shown that adult human beings may ingest 2,000-4,000 millions *S. typhi-murium* without showing symptoms other than mild diarrhœa (Hormaeche, Peluffo and Aleppo, 1936).

Savage and White (1925b) found that about 72 per cent. of food poisoning outbreaks occurred between May and October, while Pullinger and Kemp (1938) showed that *S. typhi-murium* and *S. dublin* multiplied readily in milk at 15 deg. C. They concluded that the important factor in the development of milk-borne epidemics was the ability of the organisms to develop rapidly during storage.

It follows, therefore, that the hygiene of milk production during the summer months is the main factor governing the number of outbreaks of milk-borne food poisoning. If the technique of production is good and the milk is not exposed to temperatures at which salmonellas are able to multiply, there would appear to be little danger of human infection. On the other hand, given gross contamination of the milk and exposure to temperatures permitting of bacterial multiplication, then outbreaks of disease may follow.

Summary

Bovine salmonellosis was diagnosed on 70 farms in West Wales during the two years, 1946-47. In both years the great majority of cases occurred during the warmer months of the year, i.e., June-October, inclusive. Affected animals on 67 farms were over one year old; all except one animal were females. On one farm the affected animals were nine months old. Calves were affected on the two remaining farms. Specimens from affected animals on 66 farms yielded *S. dublin* and on four farms *S. typhi-murium*. On 50 farms one animal only showed

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clinical symptoms but on 18 farms several animals were affected either concurrently or at intervals. The mortality rate is estimated to be approximately 70 per cent. Fifty-three of the outbreaks occurred on farms in the county of Carmarthen.

S. dublin bacteræmia may occur in calves a few days old but only in herds in which adult carrier animals are present. Such calves do not develop diarrhœa. Older infected calves of 7 days to 3½ months show an initial fever followed by dysentery. Enlargement of the carpal and tarsal joints occurs in some cases. As judged by the examination of fæces, calves recovered from clinical infection do not remain carriers of the organism. A more chronic form of disease, characterised by persistent diarrhœa and wasting, may be met in calves of 6-7 months of age.

In infected adult cattle an initial fever is accompanied by depression, inappetance and decreased milk yield. During this phase the organism is present in the blood and milk. This stage is followed within 24 hours by dysentery and the organism can readily be isolated from the fæces. The majority of affected animals die within 1-5 days of the onset of symptoms. Most affected in-calf animals abort. A few recover after a protracted convalescent period but remain carriers. Atypical cases occur, some of which also give rise to the carrier state. The serum agglutinins rise during the course of the disease and reach a diagnostic level by the eighth day of illness. *Post-mortem* examination shows a muco- or hæmorrhagic enteritis. A differential diagnosis is necessary between salmonellosis and other conditions characterised by similar symptoms, e.g., coccidiosis, bracken poisoning, and chilling. Field observations show that the carrier state can persist for at least two years. A point of public health importance is that small numbers of salmonella may be found in the milk of carrier animals.

A number of case records show that carrier cows transmit infection to calves, both *in utero* and post-natal. It is generally impossible to trace the source of infection when adult cattle develop clinical symptoms. The finding that although only one animal in a herd may show signs of illness, several animals may be affected sub-clinically, suggests a common source of infection.

The use of desoxycholate citrate agar (D.C.A.) is advocated as a selective medium for the isolation of salmonella from bovine fæces. As a liquid enrichment medium selenite broth (S.B.) has proved of value for the isolation of the organisms from fæces, blood clots and milk. Variants of *S. dublin* isolated have included non-motile, mucoid and non-gas producing (anærogenic) strains. Strains may also show individual fermentative differences.

The majority of normal adult cattle have serum agglutinins to *S. dublin* but the flagellar titre is not normally higher than 1/160 or the somatic titre above 1/40. At the commencement of illness, the blood titres of affected animals are within the range of such normal agglutinins. The titre rises during illness, reaching its peak about the tenth day. The agglutinins persist at a high level in the recovered animal. For the detection of such recovered but carrier animals, the use of flagellar antigen is recommended as being more likely to give a clear result than an examination for somatic agglutinins. A flagellar titre greater than 1/320 can

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be accepted as a positive diagnosis of the carrier state and a titre lower than this as negative.

The serological reaction of calves to infection differs from that of adult cattle. Very young calves, while able to produce flagellar agglutinins, appear unable to produce somatic agglutinins, while somewhat older calves respond feebly to somatic antigen. It has not been possible to determine the age when calves are capable of producing somatic agglutinins. In outbreaks of infection in calves, therefore, it is essential to use a flagellar antigen when testing sera for agglutinins.

It has been shown conclusively that transmission of infection from adult carrier animals to calves takes place. Although calves, recovered from infection, do not subsequently excrete the organism in the fæces, it is probable that in some animals the organism localises in some organ or tissue. The examination of adult animals entering an abattoir for slaughter shows that "normal" cattle may be carrying *S. dublin* or *S. typhi-murium*. *S. dublin* has also been recovered from six out of 30 farm rats examined.

Post-mortem examination of a carrier cow showed that the residual foci of infection included the gall bladder, spleen and all the lymph glands draining the liver and gastro-intestinal tract.

Four herds containing carrier animals have been kept under observation for two years. Transmission of infection occurred to calves but on three farms no clinical cases of illness appeared in adult cattle. On the fourth farm no adult animal was clinically affected for 22 months: a fatal case then occurred in a newly-introduced cow.

S. dublin can survive for long periods in fæces and water. Fæces exposed on pasture have yielded the organism after 73 days during the winter and 119 days in summer, while in contaminated water viable organisms were still present after 87 days.

The relationship between bovine salmonellosis and outbreaks of food poisoning in the human population is discussed.

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RECENT DEVELOPMENTS IN IMMUNITY, MORE PARTICULARLY FROM THE STANDPOINT OF VETERINARY MEDICINE, AND WITH ESPECIAL REFERENCE TO EGYPTIAN CONDITIONS*

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Introduction

THE problem of immunity is far from being resolved. A great many aspects of the phenomenon are still quite obscure in spite of the massive output of literature upon it. At the present time, not only are there available several text-books dealing wholly or in part with the subject, but there are several first-class periodicals. Journals such as the *Journal of Immunology* and the *Zeitschrift für Immunitätsforschung* are devoted solely to immunity, while several journals dealing with infectious disease (such as the *Journal of Infectious Diseases*), bacteriology (such as the *Journal of Bacteriology, Centralblatt für Bacteriologieusw, Annales de l'Institut Pasteur*), pathology (such as *Virchow's Archiv, Journal of Pathology and Bacteriology*), experimental pathology (such as the *Journal of Experimental Medicine, British Journal of Experimental Pathology*), and comparative pathology (such as the *Journal of Comparative Pathology and Therapeutics*) are devoted in very large part to recording phenomena which have some bearing on immunity.

Not only does an understanding of developments in our knowledge upon immunity demand at least a full acquaintance with this rapidly growing and difficult literature, but also its import is meaningless to anyone who is not himself fully conversant with the trend of the recorded information through daily practice in experimental work on the subject. Otherwise, partial knowledge becomes exceedingly dangerous inasmuch particularly as there is a tendency always on the part of the untrained mind towards over-simplification of what may be extremely complicated phenomena. Inasmuch as the approach towards the subject by the writer of an essay upon recent developments must naturally be conditioned always by his own particular experience, the exposition given here is limited in scope more particularly to the aspects mentioned in the title of the essay.

Comparison between Immunisation against Cholera in Human Beings and Cattle Plague in Cattle as Illustrating certain Essential Differences between Immunisation with an Inactivated Immunogen and an Active or "Live" Immunogen

Perhaps, in an essay of this kind, our meaning can be best made clear if we take as examples two methods of immunisation which have recently been extensively practised in Egypt, (A) the Immunisation of Human Beings against Cholera and (B) the Immunisation of Cattle against Cattle Plague.

The methods adopted present great differences. Examination of the two procedures shows a succession of reactions within the inoculated subjects which are of a different order, though in each case the objective reached is the rendering

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of the inoculated subject "resistant towards," or "immune against," a specific disease—in one case, for a relatively short period; in the other case, for the subject's lifetime, if, indeed, the subject has really undergone the desired reaction after the inoculation.

We may therefore, in the first place, summarise very briefly the succession of events which take place in the inoculated subject following upon each of the above two immunisations which we have taken as our examples:—

(*A*) *Immunisation of Human Beings against Cholera*

(*i*) The material used for inoculation (the ***antigen***, or ***immunogen***) is ***inert*** or ***inactivated***. It consists of a culture of the specific causal microorganism of cholera—the cholera vibrio—which has been killed, so that it cannot possibly afterwards proliferate within the inoculated subject. The killing is performed, however, so as to produce the least possible change in the constitution of the living organism—a satisfactory method being the addition of a very low concentration of formalin to a suspension of the cultivated organisms.

(*ii*) After inoculation, the immunity does not become effective for several days, perhaps about 8 or 9 days in most reacting subjects.

(*iii*) Before immunity becomes effective, the resistance of the subject undergoes certain fluctuations: it is not a steadily developing upward process. Long ago these fluctuations in the establishment of an immunity were noted carefully by Ehrlich, but it is only recently that their full significance has been appreciated.

(*iv*) The first fluctuation from the normal in the susceptibility of the subject towards natural infection is, as generally with this class of immunogen, that the subject is rendered still more susceptible to infection. In other words, it passes through a *Negative Phase*. This phase may be transitory, lasting only a day or so, or it may extend to about three days.

(*v*) After the normal resistance of the subject has been lowered, it afterwards steadily rises, until it soon is above the level of the normal resistance and then reaches well above it. In other words, the immunity now passes into a *Positive Phase*.

(*vi*) Often, and this is said to be the case particularly in cholera immunisation, the resistance of the subject does not reach a satisfactorily high level when the positive phase now mounts to its summit. A degree of resistance sufficiently strong to ward off natural infection is attained usually only when the immunity is reinforced by subjecting the inoculated individual to a second inoculation with the immunogen at the moment when it is estimated that the positive phase following upon the first inoculation has about reached its peak.

(*vii*) Further fluctuations have been noted, but, in any event, the immunity, after it has become well established, does not remain steady. It wanes, declines, or fades away gradually, so that in a few months the inoculated subject will have lost the immunity it had artificially acquired.

(*B*) *Immunisation of Cattle against Cattle Plague*

(*i*) The material now used in Egypt is ***active***—it must ***not*** be inactive, or inert. It consists of the virus of Cattle Plague which has undergone mutation by prolonged passage in a species of animal other than cattle, the species that has

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been found most suitable being the goat. After adjustment to the new species, the virus becomes attenuated for cattle, and the degree of attenuation can be regulated to some extent by the number of passages which the virus undergoes through goats.

(*ii*) After inoculation of the goat-adapted virus into an ox, the reaction which follows varies greatly in intensity, but whenever it occurs it is always of the same kind, namely, essentially it represents an actual attack of cattle plague.

(*iii*) The ideal reaction is one in which the goat virus obtains a foothold quickly in the tissues of the ox but the clinical symptoms never become so severe as to endanger the life of the animal.

(*iv*) All gradations in severity of the reaction may, however, be expected. In some cases, either through the normal threshold of resistance of the ox being very high or through the goat virus (assuming that it is fully active) having become too much attenuated, the goat virus does not establish itself at all in the tissues of the ox: the ox afterwards remains as susceptible as before to natural infection. In cases at the other extreme, where either the normal threshold of resistance of the particular ox is very low or the goat virus has not been sufficiently attenuated by passage, the inoculated ox may exhibit symptoms which are almost as alarmingly fatal as those of natural cattle plague. The causes of these great variations need particular amplification later.

(*v*) Assuming that the reaction evoked is ideal, or somewhat in excess of the ideal, then immunity against natural infection may be expected to take place ***very quickly***. In this respect it curiously resembles the kind of immunity which develops after the injection of an adequate dose of the specific anti-serum, namely a Passive Immunity, where resistance against attack is said to be developed immediately: for, after inoculation with the goat virus, cattle have been found to be comparatively resistant to inoculation with the natural bovine virus in about 48 hours. The mechanism of the rapidly developing immunity, however, appears to be greatly different from that of a Passive Immunity, and it arises through what has now become known as an Interference Phenomenon, which needs to be discussed later.

(*vi*) However severe the reaction is that takes place in the cattle inoculated with the goat virus, these cattle are unable to transmit the infection to healthy cattle kept in their immediate vicinity. Through prolonged passage in goats, the virus has acquired the property of spreading easily from goat to goat, but it has lost the property of spreading from cattle to cattle—or from cattle to goats.

(*vii*) The immunity set up against natural attack shows no sign of fading for such a long time that in most cases it appears to be of lifelong duration. However, in some cases, as in very young animals, it may become weakened within about two years.

What has been said above in summary by way of examples refers only to two particular host-immunogen reaction complexes, namely (A) Human subject—Cholera Vibrio and (B) Bovine Subject—Cattle Plague Virus. Present-day experience shows it would be highly dangerous to generalise from either the one or the other example.

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For example, as illustrating the variation in host reaction to a given immunogen, we may take the differing effects of inoculation with an inactivated foot-and-mouth disease virus upon the ox, the guinea-pig, and the domestic pig. With a suitable inactivated foot-and-mouth vaccine, the ox can be fairly readily immunised so as to obtain an immunity which lasts several months. The guinea-pig, after similar inoculation, develops an immunity which lasts only a few weeks. Whereas the pig never develops any satisfactory level of immunity. The species reaction towards a given immunogen therefore may vary considerably.

Again, if we take any species of animal, say the ox, we find it can be immunised readily and usefully against pasteurellosis (hæmorrhagic septicæmia) by means of an inactivated vaccine, whereas it cannot be immunised at all against tuberculosis by means of an inactivated vaccine. All sorts of gradations in the prospects of conferring immunity are now well known to occur depending upon the particular disease against which it is desired to immunise that same species.

Why it is fairly easy to immunise with the specific inactivated immunogen against one disease while it is difficult or impossible to immunise against another remained obscure until quite recently when it seems to have been shown, with the advance of protein chemistry, that it is a question of establishing the essential immunogenic constituent of the vaccine protein by arresting it locally for a sufficiently long time before it is destroyed or excreted so as to give the tissues time to elaborate immune antibodies.

Thus, generally, in the case of immunogens prepared by activation from particular groups of bacteria, such as the Mycobacteria and Brucella, the immunogenic constituents of the inactivated bacterial protein are not linked to sufficiently strongly, or adsorbed on to, less easily absorbable and excretable proteins comprised within the total bacterial protein. This point needs further discussion at a later stage in this essay.

We have thus fair uniformity in immunity response to all members comprised within a particular genus of bacteria. Hence, diseases caused by all species of Mycobacterium are impossible to immunise against by means of inactivated vaccines. On the contrary, diseases caused by all species of Pasteurella can be immunised against very well by using similar methods.

After the above introduction, we can now deal more particularly with the sequence of phenomena as they were narrated in the above two examples. For, minute examination of the phenomena as presented by these particular examples brings into contemplation most of the advances that have been made recently in our understanding of the mechanisms of immunity.

The Characteristics of the Immunogen in an Inactivated Vaccine which enable it to Evoke a Good Immunity

In the example *A* above, the immunogen was the cholera vibrio obtained in culture and inactivated by a low concentration of formalin (about 0.1 per cent.).

Recent research has shown that in the case of some groups of organisms a matter of prime importance is the variational state of the organisms. In the case of some groups, such as Clostridium, it has been shown that the flagellar (H) antigen is useless in producing immunity, whereas the somatic (or "O") antigen

is essential. In another group, Salmonella, the H antigen is certainly useless and according to the researches of Felix the O antigen is also a feeble immunogen if the culture employed is one that has, for some reason or another, lost its original high virulence through prolonged cultivation. To obtain a satisfactory immunogen in the case of this group it is necessary to preserve the very labile constituent ("Vi") of the highly virulent organism. The preservation of this constituent does not seem to be necessary, as was originally thought, however, in the case of another group, Pasteurella.

Another striking advance in our knowledge upon the effects of variational changes has been derived particularly from the study of the specific immunity responses towards the four different types of Pneumococcus. Until it was discovered that there were four entirely different immunogenic types of Pneumococcus it was difficult to understand why a solid protection could sometimes be set up against Pneumococcus pneumonia by inoculation with an inactivated Pneumococcus vaccine, whereas, in other outbreaks, inoculation with the same vaccine seemed to be powerless to ward off natural infection. The specificity of each of these types has now been clearly shown to reside in differences in the chemical composition of the capsule in the case of each type, and, chemically, the specific constituent of the capsule has been proved to be a carbohydrate. Inoculation of the purified carbohydrate does not, however, provoke any immunity response. An immunity response does not arise unless the carbohydrate is linked to another complex molecule which, in itself, is not specifically immunogenic. This non-specific linkage molecule, or group of molecules, is to be found, in the case of the inactivated vaccine, in the protein comprised within the body (or *soma*) of the Pneumococcus cell. It is not, however, absolutely essential for the specific carbohydrate to be linked on to somatic protein. Artificially, the purified carbohydrate can be linked on to several proteins derived from other sources, or even on to certain non-protein materials, and then act as an efficient immunogen.

This phenomenon of "linkage" is now understood to be a very widespread one, both in immunity and allergy (which is now believed to be, essentially, merely a phase in the development of immunity, as will be discussed later). The immunogen, or the allergen, has been proved as in the case of the Pneumococcus immunogen, to be comprised, throughout a very wide range, of a specific constituent, which in the purified state is not immunogenic or allergic, *linked on to* a non-specific grouping or substance, which arrests the former for a sufficiently long time in the tissues for the specific immunity or allergic responses to be evoked.

Capsule formation is now well known to be an acquired characteristic in the case of a large variety of microorganisms. In artificial culture, on the surface of media, the presence or absence of a capsule causes variation in macroscopic appearance of the colonies. Where capsule formation is well marked, the colonies tend to be smooth (S); where is is not marked or has been lost, the colonies tend to be rough (R). Often there is a distinct relation between these macroscopic variations and the virulence of the microorganism, S colonies tending to be virulent, and R colonies avirulent.

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An exception appears to be presented in the case of the anthrax bacillus, where the originally virulent bacilli give rise to colonies which have a ground-glass appearance, while after undergoing attenuation following on prolonged cultivation, at a temperature too high for sporulation to take place, the colonies assume a glistening, dewdroplike appearance.

The existence of immunologically different types within a definite species of microorganism is now known to be a fairly commonplace phenomenon among bacteria. Among the pathogenic protozoa, it is a still more striking phenomenon. For example, in the case of the trypanosomiases, where, after infection, the host subject exhibits a succession of crises or relapses, it has been proved that at each relapse the trypanosomes acquire different immunological characteristics. Among the viruses, splitting into types is also striking in the case of some viruses such as those of human influenza, foot-and-mouth disease and South African horse sickness, whereas in the case of some other viruses, such as most of those belonging to the variola-vaccinia group and yellow-fever, mutation of this kind does not readily take place.

It is obvious that type-variation, when it takes place within a specific pathogen, greatly complicates the practice of immunisation whether by means of the inactivated antigen or by means of an active attenuated derivative of the originally virulent strain of pathogen.

Another aspect of immunity in which the characteristics of the immunogen play an important part is that which is concerned with the property of the pathogenic microorganism to exert its effects within the infected host either in virtue of the capacity of the microorganism to proliferate rapidly and exert a destructive effect direct upon the tissues or in virtue of its capacity to produce a toxin which escapes into the body system from the site of infection. Long well-known examples of the latter kind are those of diphtheria and tetanus. An enormous amount of work has been done to improve upon the methods which had become established procedure barely twenty years ago, and which consisted essentially in the inoculation of the infected subject with antiserum ("antitoxin") derived from a hyperimmunised animal.

In current clinical practice, "antitoxin" obtained widespread use in the treatment of human diphtheria, and the efficacy of the treatment seemed to depend very largely upon early application, before the toxin had caused irreparable damage to the cells of the nervous and other systems. Therapeutic treatment of tetanus by means of antitoxin was, on the whole, not nearly so successful, but, nevertheless, tetanus antitoxin had proved extremely valuable as a preventive to tetanus arising in men or animals suffering from wounds likely to be contaminated with tetanus bacilli if they were inoculated with a prophylactic dose soon after the infliction of the wound and before symptoms of tetanus arose.

It was very shortly after the first World War that the French veterinary worker, Ramon, at the Pasteur Institute noticed that the potency of diphtheria antitoxin obtained from the hyperimmunisation of his horses did not seem to depend upon the actual toxicity of the culture filtrates which he used for inoculating the horses. Although measures had been taken, as at other laboratories, to

obtain filtrates showing the highest possible concentration of active toxin, it was found that these filtrates retained very largely their antigenic property in stimulating antitoxin formation when their activity had been partially lost. A striking discovery was then made, namely that the active toxin preserved in large part its antigenic properties when it was intentionally rendered inactive. The means which Ramon employed to inactivate the toxin was the addition of a small percentage of formalin. Since that time formalin inactivation has come into current use for dealing with a number of other toxins and with certain viruses. To the inactiyated toxin which still preserved its antigenicity Ramon gave the name Anatoxin. Several years previously, however, the phenomenon had been noticed by Ehrlich and the term " Toxoid " applied to the toxin which had lost its toxic effects but was still capable of provoking the formation of immune bodies after inoculation into an animal. About the same time as Ramon's work, the workers at the Wellcome Physiological Laboratories, England (Glenny and others) had been busily investigating the possibilities of evoking an active immunity against diphtheria, and their work was mainly concerned with exploiting the efficacy of toxin-antitoxin mixtures. Difficulties were experienced in safe balancing of the mixtures, which were overcome in part by choosing the intradermal route of inoculation and immobilising the toxin by using it adsorbed on to the precipitate formed by adding a suitable concentration of alum to the broth filtrate. These workers also soon came to realise, almost simultaneously with Ramon, that the antigenicity of the filtrates was not wholly lost when they ceased to be actively toxic, and that by using the Alum-Precipitated-Toxoid (APT) the grave risks run in using the toxin itself disappeared and there was no longer any need to employ antitoxin to neutralise the toxicity of the original toxin.

Valuable information regarding the efficacy of APT in the active immunisation of children against diphtheria was readily forthcoming from the application of a skin test, known as the Schick test. Inoculation intradermally of minimal doses of toxin into highly susceptible individuals had been known to produce a marked local reaction, whereas in resistant individuals this reaction did not arise, apparently because enough circulating antitoxin had been elaborated in their bodies to neutralise the inoculated small dose of toxin. Proof of the efficacy of immunisation with APT was therefore obtained soon by the conversion of child populations from partially Schick positive groups to wholly Schick negative groups.

This very recent development in our knowledge of immunity has proved to be one of the greatest boons conferred upon the human race, and, in progressive countries, immunisation in the above way has become propagated officially and been made compulsory.

In a similar way, tetanus toxoid has been employed, especially in war time, to prevent the onset of tetanus following war wounds, both among human beings and horses.

A most striking parallel to the above developments is that represented by the methods now employed to control diseases caused by sporulating pathogenic

anærobes, namely of the Clostridium group. The species in which the newer knowledge has proved of the greatest utility has been the sheep.

It has long been known, since the beginning of the Pasteurian era, that a powerful immunity could be set up against diesase caused by, at any rate, one member of this group, namely Blackquarter (or Blackleg) in cattle and sheep. The immunity was set up by inoculating cattle in affected areas, just before the onset of the endemic season with material in the shape of diseased muscle tissue, excised from an infected animal, that had been subjected to heat treatment so as to destroy all but a residual proportion of the contained bacterial spores. Some degree of risk was run in adopting this method, for, on the one hand, the heat treatment might not have been sufficient, so that the inoculated animals afterwards contracted fatal blackquarter; while, on the other hand, the heat treatment might have been excessive, in which case the spores would have been all killed, and the animal would remain susceptible to natural attack. To ensure establishment of living spores, two doses were recommended in the original methods, the dried infected muscle powder for the first dose being heated at a higher temperature than that for the second dose and an interval of a few days allowed to elapse between the two doses.

In the actual procedure of immunisation against blackquarter itself there have been several notable developments. Firstly, taking as the starting point in manufacture of the immunogen the diseased muscle tissue of an infected animal, it was found by the Japanese workers (Nitta and others) just before the first World War that washed spores derived from the tissue were unable by themselves to obtain a foothold in the tissues of an inoculated animal and set up disease. If, however, these washed spores were suspended in some of the flluid expressed from the muscle they readily obtained a foothold and set up fatal disease.

The filtered fluid expressed from the muscle contained no demonstrable toxin and so it was presumed that it acted upon the spore in such a way as to make them "aggressive" in the tissues of a healthy animal in virtue of containing a hypothetical substance which was called Aggressin. Immunisation of cattle with the so-called Aggressin alone, in the shape of the filtered fluid expressed from diseased muscle, was afterwards found to evoke a very satisfactory immunity, and the risk run by inoculating material containing an access of viable spores was eliminated.

Simpler and less costly ways of producing a satisfactory immunogen against this disease were, however, soon forthcoming, with the rapid improvement of anærobic technique and skill in investigating disease caused by the pathogenic anærobes, our knowledge upon which was really primitive during the period before the first World War. After satisfactory methods had been developed for growing the anærobes readily in pure culture, whether in liquid culture or on the surface of solid media, artificial cultures, instead of actually diseased muscle, could be employed as the source of the immunogen, in the case of the causal organism of blackquarter (*Clostridium chauvœi*) as well as of the other pathogenic anærobes. In the case of the particular anærobe, *Cl. chauvœi*, it was found, as had been long suspected, that it did not exert its pathogenic effects

in virtue of elaborating an exotoxin. The whole of its virulence resided in the body of the bacterium: in other words, the immunogen had perforce to consist of the somatic antigen. In much the same way, this was found to apply to the causal organisms of braxy (or bradsot) in sheep (*Vibrion septique* or *Clostridium œdematis maligni*) in north-western European countries and to those of Black Disease in sheep (*Clostridium œdematiens*) in Australia. The well-washed spores of any of these organisms were incapable of setting up disease on inoculation into the tissues, and, furthermore, none of them set up disease when administered by the mouth in the form of pure cultures.

The question remained whether the hypothetical Aggressin was really necessary to enable the organisms to obtain a foothold. In the case of Black Disease it was indeed found that the tissues of healthy sheep in infected localities could be found to harbour fully virulent spores of *Cl. œdematiens*. What was necessary for the organisms to obtain a foothold in a primary focus—which in this disease was the liver—was trauma, and the trauma was effected by the migration of immature flukes (*Fasciola hepatica*) through the liver. The spores of *Cl. œdematiens* therefore, which were already present in very small numbers in the liver, would obtain a suitable nidus for rapid proliferation in the abraded tissues, whereas they would fail to propagate so rapidly as to overcome the host's defences in the healthy tissues. (Parenthetically, a similar phenomenon had been proved to occur in the pathogenesis of "poll-evil" and "fistulous withers" in horses, where *Brucella abortus*, already present in the tissues, obtains a foothold in the lesions set up by *Onchocerca cervicalis* in the ligamentum nuchæ.)

In a very simple way, namely by injecting the washed spores of *Cl. chauvœi* suspended in a solution of calcium chloride, which was capable of abrading the tissues to the extent desired, the disease blackquarter could be set up artificially. Hence, it was unnecessary to assume the presence of the hypothetical Aggressin, the properties of which could probably be more rightly ascribed to the presence of small amounts of liberated endotoxin in the diseased tissue.

Immunisation, in the case of organisms of the type of *Cl. chauvœi*, must therefore reside in inoculation of the suitably inactivated somatic antigen (O). The flagellar antigen (H) was found to possess no immunogenic properties. Inactivation could be achieved, it was discovered, most efficaciously either by formalinisation of very young cultures, containing merely the vegetative forms of the organism, or by boiling cultures containing fully formed, well-developed spores (cultures which contained a preponderance of involution forms representing degenerated vegetative forms which had failed to reach the sporulating stage being useless).

Remarkable progress has been made in our understanding of the mechanisms of setting up an immunity against another group of anærobes, namely the "gas-gangrene" group, caused by *Clostridium welchii* and its variants. These are now specifically designated as the A type (or group), represented by the classical gas-gangrene organism, *Cl. welchii*; the B type, represented by the Lamb Dysentery ("L.D.") bacillus; the C type, represented by what was originally called *Cl. paludis*, the cause of the disease known as "struck" in the sheep on

the Romney Marsh in Kent, England; and the D type, represented by the organism originally known as *Cl. ovitoxicus*, the cause of "pulpy kidney disease" in sheep in Australia, England, and elsewhere.

Although all these organisms are morphologically and culturally identical, they differ from each other inasmuch as an immunity set up against any one of them may fail to protect against infection with the others, an exception being that the immunity produced against the B (or "Lamb Dysentery") type is capable of protecting against all four types.

The cause of this difference was found to be that at least five antigenically distinct toxins are elaborated by the several types, as indicated below:—

Culture types	*Toxins*				
	α	β	γ	δ	ε
A	+	—	—	—	—
B	+	+	+	+	+
C	+	+	+	+	—
D	+	—	—	—	+

The A, "gas-gangrene," organism contains only one toxin, which is common to all four types. The B, "lamb-dysentery," organism may contain all five toxins, and it never contains less than four. The C, or "struck," organism contains four toxins, while the D, or "pulpy kidney," organism contains, besides the "gas-gangrene" toxin, one more, which is common to it and the "lamb dysentery" organism.

The toxins can be differentiated by certain hæmolytic and necrotising properties which may or may not be possessed by a given type and by their lethal or non-lethal action on mice. Moreover, the antitoxic sera prepared against each type of organism shows specific neutralising properties as disclosed by intravenous inoculations of mixtures of antitoxin and toxic filtrate into mice. These effects are summarised in the following table:—

Culture types	*Antitoxic serum*			
	A	B	C	D
A	+	+	+	+
B	—	+	—	—
C	—	+	+	—
D	—	+	—	+

+ = neutralisation
— = no neutralisation

This knowledge has enabled us to devise measures of protection which have been singularly efficacious, particularly in averting the serious losses that had been occurring in some districts in Britain and elsewhere among new-born lambs. The measures taken have comprised either inoculation of lambs immediately after birth with specific antitoxin or (what is much more practicable) the inoculation

of the pregnant ewes with formalised whole cultures of *Cl. welchii* type B. Usually, two inoculations are recommended, the first being given in the autumn at any time from just before conception to about a month before lambing and the second about ten days before lambing. In the course of developing an active immunity a sufficient concentration of antitoxin is excreted by the dam in its colostrum, which is rich in serum globulins and pseudoglobulins, to set up protection in the new-born lamb against natural attack. (See also later, page 361.)

This represents a very novel form of conferring a passive immunity.

Among the pathogenic anærobes, therefore, the production of an immunity depends, in the case of some of them (*Cl. chauvæi*, "*Vibrion septique*," and *Cl. œdemations*), on response towards a somatic antigen, while in the case of others (*Cl welchii*, A, B, C, and D) it depends upon response towards specific exotoxins *as well as* towards *the somatic* antigen.

Earlier, it has been said that an active immunity can only be provoked against some specific immunogens when they form linkages with a variety of less readily absorbable, non-specific protein or other groupings.

In the case of some antigens, where their immunogenic activity is very weak or of short duration, some increase in effect can be obtained by repeated inoculations. This was illustrated in our original example, which was that of immunisation against the cholera vibrio.

Other methods have been attempted to increase or prolong the effect.

One method has been to incorporate the inactivated antigen in a non-absorbable menstruum, such as a mixture of lanoline or ethyl oleate and beeswax (2.5 or 5 per cent.). This has been tried with some bacterial antigens, such as *Brucella abortus*, where the results, though clearly in its favour, are not very striking, especially on account of the unsightly swellings produced at the site of inoculation. It has also been adopted, with conspicuous success, in the case of some inactivated (formalised) virus antigens, such as that of fowl plague, which, for the purpose, is cultivated within the developing chick embryo.

Another method has been to adsorb the inactivated immunogen on to some of the adsorbents well known to the enzyme chemist in his work on the purification of enzymes. The best-known immunogen subjected to treatment in this way is the virus of foot-and-mouth disease, which can be adsorbed on to freshly prepared, chemically pure, aluminium hydroxide, and inactivated with formalin. The alumina-adsorbate in this case provokes an active immunity which greatly exceeds that of the non-adsorbed inactivated virus both in dependability and duration. As before, adsorption on to the inert alumina causes arrest of the immunogen at the site of inoculation for a sufficiently long time for a satisfactory immunity response to be evoked from the host's tissues.

Some importance is attached in some cases to the particular culture medium in which a bacterial microorganism is grown for the production of a satisfactory inactivated immunogen. Thus, in the case of the cholera vibrio, for example, cultures are now grown on the surface of solid media and growth is arrested as soon as maximum growth is obtained so that the vaccine shall contain the minimum of disintegrating and decomposing bacteria which might otherwise

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produce toxic symptoms in the inoculated subjects. Likewise, the use of broth cultures for the preparation of cholera vaccine has been abandoned, both for the above reason and for the reason that toxic decomposition of growth substances present in the broth itself may cause complications.

The Methods Employed in the Inactivation of Vaccines

Essentially, the procedures adopted consist in destroying the capacity of a living micro-organism to grow and proliferate in the tissues or, in the case of an exotoxin, of depriving it of its capacity to produce toxic effects. At the same time, whatever agency is employed for inactivation, the properties of the living micro-organism itself or of the potent toxin to evoke an immunity response must be retained to the fullest possible degree. Otherwise, if the intensity of inactivation is pressed too far or is inadequately regulated, the capacity of the treated micro-organism or toxin to provoke immunity is abolished.

Different immunogens vary very greatly in the means found most appropriate to convert them into satisfactory inactivated vaccines.

Of the different agencies employed, physical agencies such as heat (most commonly of all) and irradiation with rays of short length such as rays from a mercury lamp and X-rays have been tried, sometimes after addition of a dye like methylene blue to increase absorption of the rays. A whole series of chemical agencies have been tried, some of which have been chosen for their oxidising action and some for their reducing action. The series include phenol, formalin, sodium nitrite, chloroform, toluol, various aromatic essential oils such as eucalyptol, and certain aniline dyes such as gentian or crystal violet. Lowering of surface tension on the micro-organism or virus particle, so as to deprive it of its viability while not causing it to change markedly in chemical configuration, has also been tried: an example is the use of saponine in inactivating anthrax bacilli.

To a large extent, the choice of any of these agencies has depended upon traditional practice, and often no thorough investigation has been carried out to ascertain the really best agent to employ in a given circumstance. Where the most ideal inactivating agent has come to be used in routine practice upon a given immunogen, final choice has been largely a matter of trial and error or pure chance.

For example, a method for conferring an active immunity against swine fever that has recently gained considerable favour is the inoculation of young pigs with the so-called Crystal Violet Vaccine. The method of manufacturing this vaccine consists in obtaining sterilely highly virulent blood from pigs at the height of the febrile reaction following artificial inoculation. Much depends upon the particular strain of swine fever virus used, for unless a very high concentration of highly active virus is present in the blood of the donor when it is bled out, the vaccine obtainable will be of poor immunising quality. To the citrated or defibrinated blood from the pig, crystal violet is added in the proportion of about 0.04 per cent., and the mixture maintained at 37-38° C. for a period of 14 days until no active virus remains in it. Afterwards, the vaccine is kept in the cold until it is used, when, at a dose of about 5 c.c., it can be inoculated into young pigs at about 2 months old to produce in them an active immunity which remains effective for

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6-8 months. Immunity of this duration, in the particular case of pigs reared on a commercial scale for slaughter at 8-10 months of age, solves very satisfactorily the economic problem, though, as will be mentioned later in this essay, a parallel form of immunisation with an activated vaccine is contra-indicated except in very special circumstances, in the case of cattle that are to be protected against cattle plague, where solid protection throughout the much longer survival of the animal is needed.

The mechanism of the inactivation of an active immunogen can be made reasonably clear if we examine closely the method employed in the preparation of the Crystal Violet Swine Fever Vaccine.

At first sight, the inactivating agency is the crystal violet dye, and this still remains a fixed idea in the minds of most practitioners. According to this idea, great merit is to be attached to the particular discovery of crystal violet as an inactivating agency excelling all other known agencies for the treatment of the highly virulent swine fever virus so as to render it perfectly safe for inoculation into healthy pigs, wherein it is capable of evoking a strong immunity.

On closer examination, we find that this simple explanation is hardly valid. Dyes of the pararosaniline series, which includes crystal violet, have long been known to exert a bactericidal action, more particularly upon Gram-positive bacteria. The Gram-positiveness of certain bacteria is now known to be caused by the presence of a pentose carbohydrate in the bacterial substance. Dyes belonging to some other series, such as the flavines, are known to exercise a similar effect.

Now, a striking characteristic of the filterable viruses is that on the whole they are highly resistant towards reagents of this class, which are capable of exercising a powerful bactericidal action. Not only are they resistant towards this class of disinfectants, but other disinfectant agents, such as alcohol and ether, have little viricidal effect, while some of the ordinary disinfectants, such as phenol, have a much smaller effect upon them than on the bacteria. From recent research, the viruses appear to be composed of high-molecular weight nucleo-proteins, bearing much general resemblance to the enzymes, such as pepsin and trypsin. Many of them, however, are not affected by tryptic digestion.

Researches carried out (Slavin) to find out most suitable disinfectants for use upon the swine fever virus have shown that none are really reliable for use upon the virus as it is present in swine fever blood. Instead, the addition of disinfectants generally to swine fever blood has the effect of prolonging its virulence, while the best method of ensuring rapid destruction is the putrefaction which ordinarily soon takes place in the shed blood.

Though the viruses as a class show a relatively high degree of resistance towards common disinfectants, they are often highly susceptible to changes in the acid-base equilibrium of the surrounding medium, a weak solution of common washing soda thus proving to be about the best disinfectant for use against them in practice. Their resistance towards physical agents varies greatly, and in this respect they differ very much from the vegetative forms of bacteria, which among themselves show little variation in resistance. Some viruses, such as the tobacco

mosaic virus, can withstand a high degree of temperature, even as in the case of that virus, 80° C. for about two hours, without losing their viability. Others, however, cannot withstand the ordinary temperatures of the laboratory, and have to be manipulated for experiment at temperatures near the freezing point of water. Many are extremely sensitive towards the oxidation-reduction conditions of the environment; some, like the virus of cattle plague, perishing rapidly in a " reducing " medium, while in a similar medium the survival of another virus (that of foot-and-mouth disease) is prolonged.

(*To be continued*)

CLINICAL ARTICLES

AN UNUSUAL CASE OF PARAPHIMOSIS IN A DOG

By NASHED HILMY BASTAWI, M.V.Sc.(Cairo)
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Subject.—Black cross-bred dog, two years old: Admitted into hospital on May 7, 1947; reported to have been suffering for four days from paraphimosis. The glans penis was very much swollen and threatened with gangrene. The appearance of the tissue suggested that the case was quite hopeless from the point of view of restoration of the glans to normal. The only procedure which seemed indicated in these circumstances was to attempt reduction by enlarging the margin of the prepuce. This operation having been done, the penis was reduced, but as soon as the animal was allowed to stand, the penis dropped out of the prepuce to the same length as before the operation. However, the prepuce was packed with sulphanilamide powder, the penis returned and supported within the sheath by applying a bandage round the abdomen, the animal being then kept as quiet as possible. Afterwards, daily dressings were applied, consisting of hot potassium permanganate compresses followed by packing the prepuce with sulphanilamide powder three times daily. On the fifth day after treatment, the inflammation began to subside, prolapse of the penis became less noticeable and the gangrenous aspect of the glans began to disappear. In 10 days the dog was out of danger, and in 15 days it was discharged as cured.

This case is recorded because, firstly, it had not been the writer's experience to obtain recovery after such an unpromising prognosis, and secondly, because simple and persistent treatment, as described above, which includes sulphanilamide therapy, now alters completely the prognosis in a case of this kind, which would have been considered hopeless a few years ago.

Acknowledgment.—I acknowledge the help given by Dr. J. T. Edwards, Visiting Professor and Temporary Dean, Faculty of Veterinary Medicine, Giza, Egypt.

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SEVERE COUGH IN A DOG FOUND AFTER RECOVERY TO HAVE BEEN DUE TO TRAUMATIC PHARYNGITIS

By NAGUIB GORGI

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Case History. — Cross-bred, eight-year-old dog: Brought to my private clinic at Heliopolis suffering from a severe cough. Examination revealed acute inflammation of the larynx and pharynx. The lungs were apparently normal on auscultation. The dog responded to symptomatic treatment, consisting of the administration of a cough mixture and inhalations, and in two weeks seemed normal.

Four weeks later the dog was returned to the clinic with a hard swelling, approximately 6 cm. in diameter, in the middle of the neck. After the application of hot fomentations and ichthyol ointment, the mass softened in 48 hours and was opened surgically so as to allow the escape of pus. On then examining the depth of the abscess, a foreign body was seen lying across it, which, on removal, proved to be a stitching needle of the kind the dog's lady owner herself had been using.

Comment.—It is clear that in this case the severe cough from which the dog had suffered six weeks previously, had been produced by the inflammation caused by the needle penetrating the mucosa of the pharynx, bringing about in that situation also severe irritation of the larynx. It is also clear, in the light of the after-event, that the symptomatic treatment employed, apparently with success, to alleviate the cough, had had in itself no beneficial effect whatsoever. The real cause of the trouble was not revealed until six weeks later, that is when all symptoms of cough had long disappeared, in the emergence of a foreign body at a site far remote from the larynx itself.

As cough is a condition only too familiar to the canine practitioner, for which remedies are prescribed following which there is usually a satisfactory outcome, this rather exceptional case is placed on record as illustrating that on occasion the relief obtained may not be due in any way to the therapeutic measures applied.

Acknowledgment.—I wish to acknowledge the help given to me by Dr. J. T. Edwards, formerly Honorary Dean of this Faculty, in the preparation of this note.

REVIEWS

Let's Learn to Ride. By Geoffrey Brooke. London: Constable & Co., Ltd. Price: 7s. 6d.

Books that are written by experts are apt to be intelligible only to other experts and boring to ordinary people. This is not the case in this small book written by General Brook. He is a recognised expert, his words and advice will be appreciated by his fellow experts, but at the same time it is written in a such simple and straightforward way that the most junior student or tyro horseman will be able to read it with interest and profit.

There is an air of authority about the book. I read chapter one, "Some Thoughts on Riding," with particular delight.

The instructions are not overloaded with detail, and the illustrations, by Pamela Searight, are excellent.

Equitation and Horsemanship. By MAJOR D. MAC. L. MACMILLAN. London: Constable & Co., Ltd. Price: 12s. 6d.

THE author spent many years as an instructor in India. When the Pony Club movement came along he took an interest in the children, and this book is the outcome of lectures which he prepared for the purpose of preparing the children for the various tests.

The author has supplemented his own knowledge by consulting the various standard textbooks, particularly those of the army, which until mechanisation ousted the horse, were so useful to the mounted branches.

The book, with its appendices, glossary and index, comprise a lucid encyclopædia which will be of great value to all those who are interested in Equitation and Horsemanship.

The illustrations and diagrams are good and add greatly to the value of the text.

Breeding Thoroughbreds. By COLONEL JOHN F. WALL, United States Cavalry. London: Charles Scribner's & Sons, Ltd. Price: 18s.

IT is a well-known fact that, when a man becomes interested in the breeding of horses, particularly racehorses, there is no retreat. It does not seem to matter if his life brings him into direct contact with the animals or not, he is doomed to become an addict.

Col. Wall is an authority, and he has produced a book which will be read and re-read not only by those directly or materially interested in the subject matter, but by countless numbers to whom the science of breeding is only a hobby based on theory. All schools of thought do not agree on this particular subject and we have no doubt that Col. Wall will not get away without comment and criticism, but we can say this, that he has produced a book which is almost a classic of its type.

It is beautifully illustrated, and these pictures alone will ensure that it is well thumbed and does not become a pensioner on the bookshelf.

NEWS

UNWELCOME "TOURISTS"*

DURING the autumn, winter and early spring of 1947-48 many complaints were received from farmers of damage done by rooks to crops and seedbeds. There is no doubt that much of this damage was done by rooks from the Continent—birds which come here in the "off" season and return to their own countries in spring to breed.

* Ministry of Agriculture and Fisheries News Service.

XUM

Exactly where do these rooks come from? As has so often been the case, the bird "ringing" scheme, organised in this country by the British Trust for Ornithology, helps us to answer the question. Here (and in many other countries), before the war and since, large numbers of birds of various species have been ringed, either as nestlings or as adult birds caught in special cage traps and set free again. The rings themselves are tiny, featherweight things of aluminium, each having a separate code letter and number so that there is no possibility of mistake in identification. One ring is attached to one leg of each bird, and there is no evidence that the rings hurt or inconvenience the birds in any way.

By means of ringing, naturalists have learned many useful things about the migratory movements of birds. The percentage of rings recovered is, of course, small, but nevertheless some remarkable results have been obtained. One needs only to consider the lapwing that was ringed in Cumberland and recovered in Newfoundland to realise the possibilities of the scheme. Anyone finding a dead ringed wild bird should send the ring, with exact details of time and place, to the Secretary of the Bird Ringing Committee, c/o The British Museum of Natural History, Cromwell Road, London, S.W.7.

As to the winter-visiting rooks, 24 foreign rooks bearing rings have been recovered in this country since 1931. Of these, 15 came from Germany, five from Holland and one each from Lithuania, Poland and Russia. One was ringed and released on the Borkum Lightship in the North Sea.

It is especially interesting to note just where, in this country, these 24 rooks were recovered, all of them between October and early March. Of the total of 24, seven were recovered in Norfolk, five in Suffolk, two in Essex, two in East Sussex, and one each in Bedford, Bucks, Gloucester, Hereford, Hertford, Kent, Lincs and East Yorks. The Gloucester rook, ringed in Hamburg in 1933, was recovered in 1942—nine years later. Since most of the complaints of winter damage have come from counties on the Eastern and South-eastern seaboard, this list of recoveries is not without significance. There is evidence that, on the whole, these immigrant rooks tend to stay in counties near our coast and that they do not penetrate far inland.

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